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<b>(54) Title:</b> IMMUNOPOTENTIATORY AGENTS  <b>(57) Abstract</b>  This invention relates to the use of a class of compounds as immunopotentiators, compositions containing such compounds and their manufacture, combinations of such compounds with anti-tumour or anti-infective drugs and the use of such combinations in the prophylaxis or treatment of such diseases arising from tumours or infections.		

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## IMMUNOPOTENTIATORY AGENTS

This invention relates to the use of a class of compounds as immunopotentiators, compositions containing such compounds and their manufacture, combinations of such compounds with anti-tumour or anti-infective drugs and the use of such combinations in the prophylaxis or treatment of such diseases associated with tumours or infections.

The principal protective function of the immune system relates to resistance to invasion by pathogens, including viruses, rickettsia, mycoplasma, bacteria, fungi and parasites of all types. Thus, improvement of immune response, particularly when depressed, enhances resistance to infection or infestation by any of the above pathogens.

A second protective function of the immune system is to resist engraftment of foreign tissue, either natural or in a foetal-maternal relationship; or unnatural as performed by the transplant physician.

A further protective function of the immune system is thought to be resistance to malignant cell development as in cancer. Immunopotentiators are used in cancer to enhance tumour rejection and to inhibit tumour recurrences following other forms of therapy.

Yet another protective function involves maintaining non-reactivity to self by positive suppressor mechanisms. In auto-immune and related disorders, immune reactivity, directed at self-antigens or exaggerated, elevated responses, are apparent and self destructive.

Each of the protective functions of the immune system can be modified by nonspecific therapy with immunopotentiators alone or in combination with other agents employed to improve resistance to or to kill the invading pathogen. In addition, specific resistance can be augmented by use of immunopotentiators in conjunction with an antigen as in a vaccine employing, for example, a virus, tumour cells, etc. This use can be to induce either specific immunity or tolerance. The latter is exemplified by use with an antigen in allergy or an auto-immune disease. Use of immunopotentiators may be either therapeutic or prophylactic; the latter is used particularly in aging, where infection, auto-immunity, and cancer are more common. The timing and route of

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administration are critical in determining whether a positive or negative response results. Any agent capable of augmenting an immune response may inhibit it depending on timing and dose; thus, under certain circumstances an immunopotentiator could be used as an immunosuppressive agent for use in allergy, auto-immunity and transplantation.

By immunopotentiator is meant an agent which is capable of restoring a depressed immune function, or enhancing normal immune function, or both. However because of certain elements of the immune system an immunopotentiator can also have immunosuppressive effects.

Immune responses are orchestrated by T-lymphocytes whose stereo-specific receptors are triggered by antigenic fragments bound to specialised molecules (MHC) on the surface of antigen presenting cells. In addition to the specific interaction between T-cell receptor and antigen-MHC complex, T-cells require costimulatory signals which are provided by antigen presenting cells. The interaction between antigen presenting cells and T-cells involves accessory adhesion molecules some of which are linked to the T-cell receptor:antigen-MHC interaction and others which are separate from this interaction. Some of these adhesion molecules provide costimulatory signals. In addition, studies made in vitro have established that transient covalent chemical reactions between cell surface ligands are essential in the antigen-specific activation of T-cells (Rhodes, J. (1989), J.Immunol. 143:1482; Gao, X.M. & Rhodes, J. (1990), J.Immunol. 144:2883; Rhodes, J. (1990), J.Immunol. 145:463). These take the form of carbonyl-amino condensation (Schiff base formation) between groups on APC and T-cell surface.

The patent specification WO 9203164 discloses a novel adjuvant, NAGO, which works by enzymatically increasing the expression of Schiff base forming ligands. The patent specification DE 3508875 discloses cinnaldehyde as an immunopotentiator in rabbits infected with Herpes PSR virus. The patent specification EP54924 discloses certain compounds of this invention, their synthesis and their properties as "left-shifters" of the oxygen-dissociation curve. All references identified herein above are hereby incorporated by reference thereto.

Therapeutic targets for an immunopotentiatory drug include chronic virus infections such as viral hepatitis and human immunodeficiency virus. Cancer is also a therapeutic

target. Evidence is accumulating to show that chronic virus infections and malignant tumours evade the immune system in various ways. Thus, although cytotoxic T-cells and antibodies are produced against abnormal cells (whether virus infected or malignant) the immune system fails to halt the pathogenic process. A common process of evasion may exist in which viruses and tumours exploit the mechanism by which non-responsiveness to self is maintained. Self-reactive T-cells are eliminated during foetal development by ligation of the T-cell receptor (TCR) in the absence of costimulatory signals. As a result such T-cells undergo programmed cell death. Self-reactive T-cells in the periphery are also maintained in a non-responsive state through ligation of the TCR in the absence of costimulation. This is termed anergy. Evasion of the immune system may occur in pathogenic processes when costimulatory signals are subverted. The result is that T-cells responding to the disease become anergic or undergo programmed cell death. There is increasing evidence for the latter in AIDS. Schiff base-forming immunopotentiators should be effective in preventing this escape mechanism because they act by providing costimulation to T-cells. In this way Schiff base-forming immunopotentiatory drugs may expressly correct the lesion in immunity that allows viral infections to persist and certain malignant tumours to progress.

It has now been found that certain small molecules that participate in carbonyl-amino condensation reactions, surprisingly act as immunopotentiators.

The present invention relates to the use of compounds or physiologically acceptable salts thereof, which form Schiff bases or hydrazones with T-cell surface carbonyl or amino groups, with the proviso that the compound cinnaldehyde excluded, for the manufacture of a medicament for the potentiation of an immune response. Hereinafter the compounds or physiologically acceptable salts thereof are referred to as "compound(s) of the invention".

The present invention relates to the use of a compound of formula (I) :



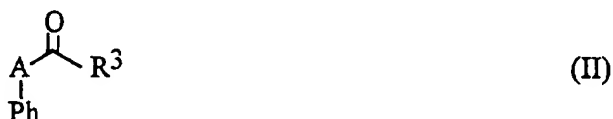
wherein R is hydrogen, methyl optionally substituted by a substituted phenyl, or by two C<sub>1-4</sub> alkyl groups and a hydroxy group;

$R^1$  is a group  $CH_2COR^2$  or the tautomeric form thereof wherein  $R^2$  is methyl optionally substituted by a group  $CO(CH_2)_xCO_2H$  wherein  $x$  is 1 to 7;

or  $R^1$  is a group A-Ph where A is O,  $CH_2$ ,  $CH(CH_3)$ , CO or a single bond and Ph is an optionally substituted phenyl group;

or R is linked to  $R^1$  to form a nine or ten-membered optionally substituted bicyclic ring system of which one ring is benzene or a physiologically acceptable salt thereof.

Compounds represented by formula (II) wherein A and Ph are as hereinbefore defined;



$R^3$  is H,  $C_{1-4}$  alkyl or  $CH_2(C_6H_4)R^4$ , wherein  $R^4$  is hydroxy, or  $C_{1-4}$  alkoxy or a physiologically acceptable salt thereof constitute a preferred sub-class of compounds within the scope of formula (I).

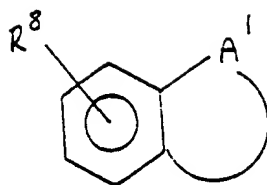
A further preferred sub-class of compounds within the scope of formula (I) are represented by formula (III).



wherein  $R^7$  is a group  $CH_2COR^2$  where  $R^2$  is hereinbefore defined or a physiologically acceptable salt thereof.

A further preferred sub-class of compounds within the scope of formula (I) wherein the bicyclic ring system is of formula (IV)


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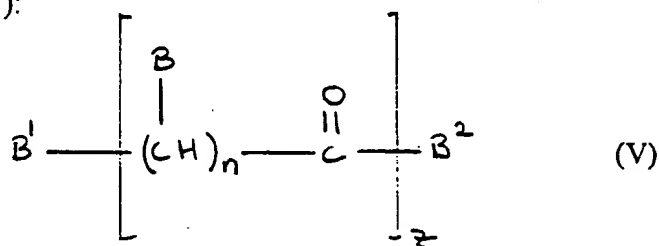
(IV)

wherein  $R^8$  is hydroxy or alkoxy;

$A^1$  is O, CO or  $CH_2$ ;

 represents  $C_{2-3}$  alkylene optionally substituted by a phenol group.

The present invention more particularly relates to the use of a compound of the formula (V):



(V)

or a physiologically acceptable salt thereof, for the manufacture of a medicament for the potentiation of an immune response, wherein

$z$  is 0 or 1;

$n$  is 0 or 1;

$B$  is hydrogen or  $C_{1-4}$  alkyl;

$B^1$  is hydrogen or an optionally substituted phenyl;

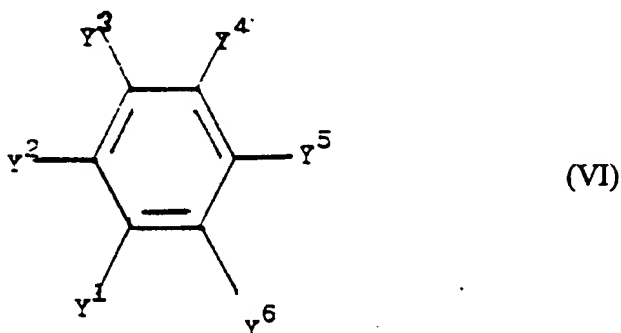
$B^2$  is hydrogen, methyl, or methyl optionally substituted by a group  $CO(CH_2)_xCOOH$  wherein  $x$  is 1 to 7, or may be linked via an oxygen or a hydrocarbyl chain optionally containing an oxygen to  $B^1$  when  $B^1$  is phenyl to form an optionally substituted bicyclic ring system containing 8 or 10 atoms of which one may be oxygen and the rest carbon,

when  $z = 0$ ;  $B^1$  cannot be hydrogen, and  $B^2$  must be hydrogen.

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When  $B^1$  is an optionally substituted phenyl preferred substituents are defined as  $Y^1$ ,  $Y^2$ ,  $Y^3$ ,  $Y^4$ ,  $Y^5$  and  $Y^6$  as defined below in formula (VI).

A preferred group of compounds of formula (I) are those represented by formula (VI):

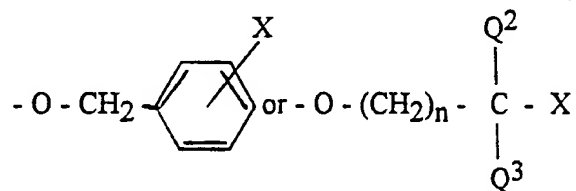


wherein  $Y^1$  is selected from hydrogen, hydroxy,  $C_{1-4}$  alkylamino, and

alkanoylamino having 1 to 4 carbon atoms in the alkyl moiety thereof;

$Y^2$ ,  $Y^3$  and  $Y^4$  are independently selected from hydrogen, halogen,  $C_{1-4}$  alkyl,  $C_{1-4}$  alkoxy, haloalkyl, hydroxyl and benzyloxy; and

$Y^5$  is hydroxyl or a group  $Q^1$  wherein  $Q^1$  is either



where

$Q^2$  and  $Q^3$  are independently selected from hydrogen and  $C_{1-4}$  alkyl;

$X$  is selected from cyano, carboxyl or a functional carboxylic acid derivative 5-tetrazolyl and alkylsulphonylcarbonyl having 1 to 6 carbon atoms in the alkyl moiety thereof; and  $n$  is 0 to 6;



$Y^6$  is  $-(C_pH_{2p})CHO$ , wherein  $p = 0$  to  $5$

when  $p$  is  $> 1$  the formyl( $-CHO$ ) in the group  $Y^6$  may be on any carbon atom and the alkylene ( $C_pH_{2p}$ ) may be linear or branched;

or  $Y^6$  is  $(CH_2CO)_q-X^1$  or  $(COCH_2)_q-X^1$  where  $q = 1$  to  $3$  and

$X^1$  is methoxyphenyl, or  $C_{1-4}$  alkyl or a physiologically acceptable salt thereof.

Compounds wherein  $X$  is selected from cyano, 5-tetrazolyl, alkylsulphonylcarbamoyl having 1 to 6 carbon atoms in the alkyl moiety thereof and a group  $-CO.Y$ , where

$Y$  is  $-OR^9$  and  $R^9$  is hydrogen,  $C_{1-4}$  alkyl or benzyl, or

$Y$  is  $-NR^{10}R^{11}$  where  $R^{10}$  and  $R^{11}$  are independently hydrogen or alkyl of 1 to 4 carbon atoms,

constitute a preferred sub-class of compounds within the scope of formula (VI).

Compounds within formula (VI) wherein  $X$  is a carboxylic acid derivative include:

pharmaceutically acceptable esters, including aliphatic and aromatic hydrocarbon esters such as alkyl and aralkyl esters where for example the alkyl has 1 to 12 and preferably 1 to 4 carbon atoms (in particular methyl, ethyl, isopropyl and t-butyl) and where the aralkyl is for example benzyl and amino acid esters (for example, L-valyl or L-isoleucyl); and

pharmaceutically acceptable amides, including the unsubstituted amide, N-substituted amides and N,N-disubstituted amides (embracing cyclic and heterocyclic amides) where the substituent group(s) is (are) for example aliphatic hydrocarbon such as alkyl, in particular  $C_{1-4}$  alkyl such as methyl, ethyl, isopropyl and t-butyl.

Compounds of formula (VI) wherein  $Y^1$  is alkylamino can form acid addition salts; in such salts the identity of the acid is of less importance although for use in medicine it is physiologically acceptable to the recipient. Suitable acids are well known in the art, for example hydrochloric acid and acetic acid.

As a subclass within formula (V) may be mentioned the use of compounds wherein

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B<sub>1</sub> and B<sub>2</sub> form a bicyclic ring system, the bicyclic ring system is selected from coumarin, tetralone, benzofuranone or naphthoquinone substituted by up to four substituents selected from hydrogen, C<sub>1-4</sub> alkoxy, hydroxy or a phenol group together with physiologically acceptable salts thereof.

Compounds of the invention include the following

compounds named in Table 2 and

5-(2-formyl-3-hydroxyphenoxy)pentanamide,

N,N-diethyl 5-(2-formyl-3-hydroxyphenoxy)pentanamide

N-isopropyl 5-(2-formyl-3-hydroxyphenoxy)pentanamide,

ethyl 5-(2-formyl-3-hydroxyphenoxy)pentanoate,

5-(2-formyl-3-hydroxyphenoxy)pentanonitrile,

(±)-5-(2-formyl-3-hydroxyphenoxy)-2-methylpentanoic acid, together with salts thereof,

5-(2-formyl-3-hydroxyphenoxy)-2,2-dimethylpentanoic acid, together with salts thereof,

methyl 3-(2-formyl-3-hydroxyphenoxy)methylbenzoate,

3-(2-formyl-3-hydroxyphenoxy)methylbenzoic acid, together with salts thereof,

benzyl 5-(2-formyl-3-hydroxyphenoxy)pentanoate,

5-[4-(2-formyl-3-hydroxyphenoxy)-N-butyl]tetrazole, together with salts thereof,

7-(2-formyl-3-hydroxyphenoxy)heptanoic acid, together with salts thereof,

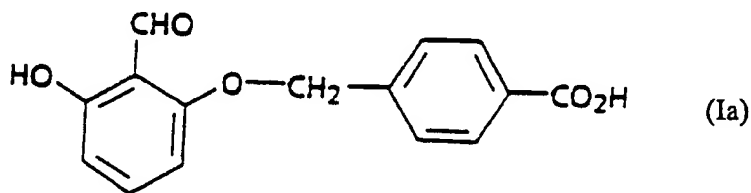
5-(2-formyl-3-hydroxy-4-n-propoxyphenoxy)pentanoic acid, together with salts thereof,

5-(4,6-dichloro-2-formyl-3-hydroxyphenoxy)pentanoic acid, together with salts thereof,

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5-(2-formyl-3-hydroxyphenoxy)-N-methylsulphonylpentanamide,  
ethyl 4-(2-formyl-3-hydroxyphenoxy)methyl)benzoate,  
5-(4-chloro-2-formyl-3-hydroxyphenoxy)pentanoic acid, together with salts thereof,  
5-(3-acetylamino-2-formylphenoxy)pentanoic acid, together with salts thereof,  
Aminoguanidine,  
4-(2-formyl-3-hydroxyphenoxy)butanoic acid, together with salts thereof,  
6-(2-formyl-3-hydroxyphenoxy)hexanoic acid, together with salts thereof,  
ethyl 4-(3-acetylamino-2-formylphenoxy)methyl)benzoate,  
4-(3-acetylamino-2-formylphenoxy)methyl)benzoic acid, together with salts thereof,  
2-(2-formyl-3-hydroxyphenoxy)methyl)benzoic acid, together with salts thereof,  
5-[4-(2-formyl-3-hydroxyphenoxy)methyl)phenyl]tetrazole, together with salts thereof,  
5-(2-formyl-3-hydroxy-4-methoxyphenoxy)pentanoic acid, together with salts thereof,  
3-(2-formyl-3-hydroxyphenoxy)propionitrile.

A particularly preferred compound is



chemically named 4-(2-formyl-3-hydroxyphenoxy)methyl)benzoic acid, together with salts thereof. The compound of formula (Ia) have been disclosed in EP00549724B.

Therefore, a compound of the invention or a physiologically acceptable salt thereof, may be used for the treatment of diseases where there is a defect in the immune system and/or an ineffective host defence mechanism, or to enhance activity of the immune system above normal levels.

In a further aspect, a compound of formula (I) or a physiologically acceptable salt thereof, may be used for the treatment of diseases where there is a defect in the immune system and/or an ineffective host defence mechanism, or to enhance activity of the immune system above normal levels.

A compound of the invention or a physiologically acceptable salt thereof may be administered for the treatment or prophylaxis of immunodeficient mammals alone or in combination with other therapeutic agents, for example, with other antiviral agents, or with other anticancer agents.

A compound of the invention or a physiologically acceptable salt thereof may be administered for the treatment or prophylaxis of immunodeficient mammals alone or in combination with other therapeutic agents, for example, with other antiviral agents, or with other anticancer agents.

By potentiation of an immune response is meant restoration of a depressed immune function, enhancement of a normal immune function, or both. Immune function is defined as the development and expression of humoral (antibody-mediated) immunity, cellular (T-cell-mediated) immunity, or macrophage and granulocyte mediated resistance.

In this specification the term "immunodeficient patient" will be used to describe patients with a deficient or defective immune system. An immunodeficient patient can be characterised by means of a T-lymphocyte proliferation assay. Using this assay immunodeficient patients are characterised by reduced ability of the T-cells to respond to stimulation by mitogens and recall antigens. An example of a mitogen and a recall antigen commonly used in this assay are phytohaemagglutinin (PHA) and tetanus toxoid respectively.

In Adler et al., Cancer (1980) 45, 2062-2063 the immune function of breast cancer patients was evaluated by means of the T-lymphocyte proliferation assay using PHA. Quantitative estimation of the lymphocyte response to PHA was based on Stimulation

index (SI). In the aforementioned paper a SI value below 14 was regarded as immunodeficient. Accordingly in this application we also consider that cancer patients in response to PHA who have a SI value below 14 are immunodeficient.

Immunodeficiency and immunosuppression is thought to occur in many clinical situations where there are lesions in signalling to lymphocytes, particularly T-cells which orchestrate the immune response. T-cells require two signals in order to initiate an effective immune response and these are:

- (i) occupation of the specific T-cell receptor for antigen, and
- (ii) stimulation through costimulatory receptors.

In the absence of signal (ii), not only do T-cells fail to respond but they may also become chronically paralysed or anergic. Persistent viral and bacterial infections and neoplastic disease can produce T-cell hyporeponsiveness by interfering in various ways with the delivery of second signals and in this way evade the immune response. The compound of formula (I) appears to work by substituting or otherwise compensating for deficient costimulatory signals to T-cells.

There is a variety of circumstances in which the immune system may be defective or deficient. Thus, for example immune system deficiency is common in immature or premature infants (neonates). It may also result from suppression by certain drugs, which may be deliberate e.g. as a side-effect of cancer chemotherapy. Disordered growth of one or more constituent parts of the immune system, e.g. as in certain forms of cancer, may also result in immunodeficiency. Immune deficiency may furthermore be caused by viral infections, including human immunodeficiency virus (HIV).

By an "effective amount" is meant the amount of a compound of the invention which will restore immune function to near normal levels, or increase immune function above normal levels in order to control or reduce infection.

A further aspect of the present invention provides a method of treating immunodeficient patients, which comprises administering to a mammal (including human) an effective amount of a compound of the invention, or a physiologically acceptable salt thereof.

According to a further aspect of the present invention provides for the use of a compound of the invention or physiologically acceptable salts thereof for the treatment and/or prophylaxis of acute and chronic viral infections.

Examples of acute viruses against which immunopotentiatory therapy with a compound of the invention or physiologically acceptable salts thereof may be used, preferably in conjunction with an antiviral agent, are:

herpes viruses, influenza viruses, parainfluenza viruses, adenoviruses, coxsackie viruses, picorna viruses, rotaviruses, hepatitis viruses, mumps virus, rubella virus, measles virus, pox viruses, respiratory syncytial viruses, papilloma viruses, and enteroviruses, arenavirus, rhinoviruses, poliovirus, Newcastle disease virus, rabies virus, arboviruses.

Examples of chronic viral infections against which immunopotentiatory therapy with a compound of the invention or physiologically acceptable salts thereof may be used are persistent herpes virus infections, Epstein Barr virus infection, persistent rubella infections, papovirus infections, hepatitis virus infections and human immunodeficiency virus infection.

The present invention is applicable to the treatment of viral hepatitis in all of its forms, five types now being recognised hepatitis A, B, C, D and E respectively.

Of the DNA viruses, those of the herpes group are the sources of the most common viral illnesses in man. The group includes herpes simplex virus types 1 and 2 (HSV), varicella zoster virus (VZV), cytomegalovirus (CMV); Epstein-Barr virus (EBV), human herpes virus type 6 (HHV-6) and human herpes virus type 7. HSV 1 and HSV 2 are some of the most common infectious agents of man. Most of these viruses are able to persist in the host's neural cells; once infected, individuals are at risk of recurrent clinical manifestations of infection which can be both physically and psychologically distressing.

HSV infection is often characterised by extensive and debilitating lesions of the skin, mouth and/or genitals. Primary infections may be subclinical although tend to be more severe than infections in individuals previously exposed to the virus. Ocular infection by HSV can lead to keratitis or cataracts thereby endangering the host's sight. Infection in the newborn, in immunocompromised patients including AIDS patients or penetration of the infection into the central nervous system, can prove fatal.

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Transmission of the virus is by direct physical contact between a host and a recipient; the spread of HSV infection is therefore considered a very significant social problem, particularly as no effective vaccine is yet available.

Varicella zoster (VZV) is a herpesvirus which causes chickenpox and shingles. Chickenpox is the primary disease produced in a host without immunity and in young children is usually a mild illness characterised by a vesicular rash and fever. Shingles or zoster is the recurrent form of the disease which occurs in adults who were previously infected with varicella-zoster virus. The clinical manifestations of shingles are characterised by neuralgia and a vesicular skin rash that is unilateral and dermatomal in distribution. Spread of inflammation may lead to paralysis or convulsions. Coma can occur if the meninges become affected. In immunodeficient patients VZV may disseminate causing serious or even fatal illness. VZV is of serious concern in patients receiving immunosuppressive drugs for transplant purposes or for treatment of malignant neoplasia and is a serious complication of AIDS patients due to their impaired immune system.

In common with other herpes viruses, infection with CMV leads to a lifelong association of virus and host and, following a primary infection, virus may be shed for a number of years. Congenital infection following infection of the mother during pregnancy may give rise to clinical effects such as death or gross disease (microcephaly, hepatosplenomegaly, jaundice, mental retardation), retinitis leading to blindness or, in less severe forms, failure to thrive, and susceptibility to chest and ear infections. CMV infection in patients who are immunocompromised for example as a result of malignancy, treatment with immunosuppressive drugs following transplantation or infection with Human Immunodeficiency virus may give rise to retinitis, pneumonitis, gastrointestinal disorders and neurological diseases. CMV infection in AIDS patients is a predominant cause of morbidity as, in 50-80% of the adult population, it is present in a latent form and can be re-activated in immunocompromised patients.

Epstein-Barr virus (EBV) causes infectious mononucleosis and hairy leukoplakia, and is also suggested as the causative agent of human cancer, such as nasopharyngeal cancer, immunoblastic lymphoma, Burkitt's lymphoma.

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HBV is a viral pathogen of world-wide major importance. The virus is aetiologically associated with primary hepatocellular carcinoma and is thought to cause 80% of the world's liver cancer. In the United States more than ten thousand people are hospitalised for HBV illness each year, and an average of 250 die with fulminant disease. The United States currently contains an estimated pool of 500,000-1-million infectious carriers. Chronic active hepatitis generally develops in over 25% of carriers, and often progresses to cirrhosis. Clinical effects of infection with HBV range from headache, fever, malaise, nausea, vomiting, anorexia and abdominal pains. Replication of the virus is usually controlled by the immune response, with a course of recovery lasting weeks or months in humans, but infection may be more severe leading to persistent chronic liver disease outlined above.

Of the RNA viruses, one group has assumed a particular importance this is the retroviruses. Retroviruses form a sub-group of RNA viruses which, in order to replicate, must first 'reverse transcribe' the RNA of their genome into DNA ('transcription' conventionally describes the synthesis of RNA from DNA). Once in the form of DNA, the viral genome may be incorporated into the host cell genome, allowing it to take advantage of the host cell's transcription/translation machinery for the purposes of replication. Once incorporated, the viral DNA is virtually indistinguishable from the host's DNA and, in this state, the virus may persist for the life of the cell.

In the case of immunosuppression resulting from HIV infection, prophylaxis may be required by those diagnosed as seropositive for HIV i.e. having antibodies to HIV, and those with PGL (progressive generalised lymphadenopathy) or ARC (AIDS-related complex) as well as patients suffering from AIDS or patients suffering from AIDS-like immune deficiencies where the HIV infection is not detectable and who also require immunorestitution by means that are not specific to any particular virus.

The compounds according to the invention may be employed alone or in combination with other therapeutic agents for the treatment of the above infections or conditions. Combination therapies according to the present invention comprise, the administration of at least one compound of the invention or a physiologically functional derivative thereof and at least one other pharmaceutically active ingredient. The active ingredient(s) and pharmacologically active agents may be administered together or separately and, when administered separately this may occur simultaneously or



sequentially in any order. The amounts of the active ingredient(s) and pharmacologically active agents(s) and the relative timings of administration will be selected in order to achieve the desired combined therapeutic effect. Preferably the combination therapy involves the administration of one compound of the invention or a physiologically functional derivative thereof and one of the agents mentioned herein below.

Examples of such further therapeutic agents include agents that are effective for the treatment of HIV infections or associated conditions such as 3'-azido-3'-deoxythymidine (zidovudine), other 2', 3'-dideoxynucleosides such as 2',3'-dideoxycytidine, 2',3'-dideoxyadenosine and 2',3'-dideoxyinosine, carbovir, acyclic nucleosides (for example, acyclovir), 2',3'-didehydrothymidine, protease inhibitors such as N-tert-butyl-dehydro-2-[-2(R)-hydroxy-4-phenyl-3(S)-[[N-2-quinolyl-carbonyl]-L-asparginy]butyl]-(4aS,8aS)-isoquinoline-3(S)-carboxamide (Ro 31-8959), oxathiolan nucleoside analogues such as cis-1-(2-hydroxymethyl)-1,3-oxathiolan-5-yl)-cytosine or cis-1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-5-fluoro-cytosine, 3'-deoxy-3'-fluorothymidine, 2',3'-dideoxy-5-ethynyl-3'-fluorouridine, 5-chloro-2',3'-dideoxy-3'-fluorouridine, Ribavirin, 9-[4-hydroxy-2-(hydroxymethyl)but-1-yl]guanine (H2G), TAT inhibitors such as 7-chloro-5-(2-pyrrolyl)-3H-1,4-benzodiazepin-2(H)-one (Ro5-3335), or 7-chloro-1,3-dihydro-5-(1H-pyrrolyl)-3H-1,4-benzodiazepin-2-amine (Ro24-7429) interferons such as  $\alpha$ -interferon, renal excretion inhibitors such as probenecid, nucleoside transport inhibitors such as dipyridamole; pentoxifylline, NAcetylCysteine, Procysteine,  $\alpha$ -trichosanthin, phosphonoformic acid, as well as immunomodulators such as interleukin II, granulocyte macrophage colony stimulating factors, erythropoietin, soluble CD<sub>4</sub> and genetically engineered derivatives thereof. Examples of such further therapeutic agents which are effective for the treatment of HBV infections include carbovir, oxathiolan nucleoside analogues such as cis-1-(2-hydroxymethyl)-1,3-oxathiolan-5-yl)-cytosine or cis-1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-5-fluoro-cytosine, 2',3'-dideoxy-5-ethynyl-3'-fluorouridine, 5-chloro-2',3'-dideoxy-3'-fluorouridine, 1-( $\beta$ -D-arabinofuranosyl)-5-propynyluracil, acyclovir and interferons, such as  $\alpha$  interferon.

It has been reported (Hughes, W.T. (1987) Treatment and Prophylaxis of Pneumocystis carinii pneumonia, Parasitology Today 3(11) 332-335) that at least 60% of patients with acquired immunodeficiency syndrome (AIDS) suffer from Pneumocystis carinii pneumonia.

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Without treatment, Pneumocystis carinii pneumonia is almost always fatal in immunocompromised hosts. The most widely used treatments for this condition are trimethoprim-sulphamethoxazole (cotrimoxazole) and pentamidine. However, both of these treatments have been reported to be only around 50-70% effective in AIDS patients and to produce a much higher than usual incidence of adverse reactions (about 50%) (Wofsy, C.B. Antimicrobial Agents Annual, 1986, Vol 1, p377-400). There is thus a need for new agents, especially for the prophylaxis of P.carinii pneumonia.

In another aspect the present invention provides the use of a compound of the invention or a physiologically acceptable salt thereof for the manufacture of a medicament for the treatment and/or prophylaxis of Pneumocystis carinii infections in mammals (including humans).

In a yet further aspect the present invention provides for the use of a compound of the invention or a physiologically acceptable salt thereof to treat conditions resulting from relative T-cell deficiencies such as DiGeorge Syndrome, fungal infections, mycoplasma infections, tuberculosis, leprosy, and systemic lupus erythematosus.

In another aspect of the present invention provides for the use of the compound of the invention or a physiologically acceptable salt thereof for the manufacture of a medicament for the treatment and/or prophylaxis of cancer in mammals (including humans).

In a further aspect of the present invention provides for the use of the compound of the invention or a physiologically acceptable salt thereof for the manufacture of a medicament for the treatment and/or prophylaxis of cancer other than in radiotherapy.

A compound of the invention or physiologically acceptable salts thereof can also be used for the treatment and prophylaxis of cancer, at a daily dosage substantially lower and for a longer duration than the dosage previously disclosed in EP54924. The duration of dosing for the treatment and prophylaxis of cancer using a compound of the invention is longer than would be required for radiosensitisation or other forms of tumour oxygenation.

Examples of forms of cancers particularly suitable for treatment with a compound of the invention are : melanoma, cervical cancer, breast cancer, colon cancer, cancer of the head and neck, gastric cancer, renal cancer, laryngeal cancer, rectal cancer, and

non-Hodgkins lymphoma. Cancers that express tumour specific antigens or antigens rarely expressed or expressed at very low density on normal cells, are likely therapeutic targets. Cancers which contain tumour specific cytotoxic T-cells which are anergic or otherwise ineffective are likely targets for therapy. Surgically resected tumours where there is a high risk of recurrence are also suitable for therapy with a compound of the invention. Also early stage cancer patients with minimal disease or localised disease are suitable for therapy.

Compounds of the invention are thought to act by providing a co-stimulatory signal to cloned (partially) activated T-cells in vitro, thus maximally activating T-cells.

A further aspect of the present invention provides for the use, as a vaccine adjuvant, of a compound of the invention or physiologically acceptable salts. A vaccine may therefore be prepared by formulating the antigenic component with a compound of the invention.

Compounds of the invention may be administered to the human recipient by a route selected from oral, parenteral (including subcutaneous, intradermal, intramuscular and intravenous), rectal and inhalation. The size of an effective dose of a compound will depend upon a number of factors including the identity of the recipient, the type of immunopotential involved, the severity of the condition to be treated and the route of administration, and will ultimately be at the discretion of the attendant physician.

For each of the aforementioned conditions, such an effective dose will generally be in the range 0.5 to 50 mg/kg bodyweight of human recipient per day, preferably in the range 0.1 to 20 mg/kg bodyweight per day and most preferably in the range 1 to 10 mg/kg bodyweight per day; an optimum dose is 3 mg/kg bodyweight per day. The above doses are for human usage.

The desired dose may be presented as between two and four sub-doses administered at appropriate intervals throughout the day. Thus where three sub-doses are employed each will generally lie in the range .03mg to 33mg, preferably 0.16mg to 166mg and most preferably 0.3 to 6.6mg (acid)/kg bodyweight with an optimum of 1.0mg (acid)/kg bodyweight. A daily dose for a human weighing of the order of 50 kg will thus generally lie in the range 5mg to 5g (acid), preferably in the range 25mg to 2.5g (acid) and most preferably in the range 50mg to 1g (acid). Optimally a human daily

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dose is 150mg (acid). The desired dose is preferably presented as a daily dose over a period of at least 5 days, most preferably over a period of at least 28 days.

While it is possible for the compounds of the invention to be administered as the raw chemical it is preferable to present them as a pharmaceutical formulation preparation. The formulations of the present invention comprise a compound of the invention, as above defined, together with one or more acceptable carriers therefor and optionally other therapeutic ingredients. The carrier(s) must be 'acceptable' in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The formulations include those suitable for oral, parenteral (including subcutaneous, intradermal, intramuscular and intravenous) and rectal administration although the most suitable route may depend upon for example the condition of the recipient. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association a compound of the invention (the active ingredient) with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired formulation.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression, or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

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The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein.

Formulations for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain antioxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Formulations for rectal administration may be presented as a suppository with the usual carriers such as cocoa butter.

EP54924 contains no invitation to administer the compound of formula (Ia) by the nasal or pulmonary route nor any suggestion that the said compound, if administered in such a manner, would be effective in the treatment of the conditions therein taught; the said disclosure likewise contains no description of any formulation suitable for administration by the nasal or pulmonary route.

Formulations suitable for pulmonary administration via the buccal cavity are presented such that particles containing the active ingredient and desirably having a diameter in the range 0.5 to 7 microns are delivered into the bronchial tree of the recipient.

As one possibility such formulations are in the form of finely comminuted powders which may conveniently be presented either in a pierceable capsule, suitably of for example gelatin, for use in an inhalation device, or alternatively as a self-propelling formulation comprising active ingredient, a suitable liquid propellant and optionally other ingredients such as surfactant and/or a solid diluent. Self-propelling formulations may also be employed wherein the active ingredient is dispensed in the form of droplets of a solution or suspension.

Such self-propelling formulations are analogous to those known in the art and may be prepared by established procedures. Suitable they are presented in a container

provided with either a manually-operable or automatically functioning valve having the desired spray characteristics; advantageously the valve is of a metered type delivering a fixed volume, for example 50 to 100 microlitres, upon each operation thereof.

As a further possibility the active ingredient may be in the form of a solution for use in an atomiser or nebuliser whereby an accelerated airstream or ultrasonic agitation is employed to produce a fine droplet mist for inhalation.

Formulations suitable for nasal administration include presentations generally similar to those described above for pulmonary administration. When dispensed such formulations should desirably have a particle diameter in the range 10 to 200 microns to enable retention in the nasal cavity; this may be achieved by, as appropriate, use of a powder of a suitable particle size or choice of an appropriate valve. Other suitable formulations include coarse powders having a particle diameter in the range 20 to 500 microns, for administration by rapid inhalation through the nasal passage from a container held close up to the nose, and nasal drops comprising 0.2 to 5% w/v of the active ingredient in aqueous or oily solution.

Preferred unit dosage formulations are those containing an effective dose, as hereinabove recited, or an appropriate fraction thereof, of the active ingredient.

The following Examples are provided in illustration of the present invention and should not be construed as in any way constituting a limitation thereof. All temperatures are in degrees Celsius (°C).

#### Pharmaceutical Formulations

##### Solution for nebulisation

Compound of the invention	1.0 mg
Water for injections	to 10.0 mL

Dissolve the compound of formula (I) as for injections. Sterilize the solution by passage through a membrane filter, 0.2µm pore size, collecting the filtrate in a sterile receiver. Fill into sterile glass ampoules, 10mL/ampoule, under aseptic conditions and seal each ampoule by fusion of the glass.

### Self-propelling formulation

**Compound of the invention, micronised** 1.0 mg

**Propellant** to 5.0 mL

Suspend the micronised compound of formula (I) in the propellant. Fill this suspension under pressure into preformed, valved aerosol canisters, 5mL/canister, through the valve orifice.

The propellant is a commercially available mixture of trichloromonofluoromethane, dichlorodifluoromethane and dichlorotetrafluoroethane.

### Powder for inhalation

Compound of the invention, micronised	1.0 mg
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**Lactose** 29.0 mg

Triturate and blend the micronised compound of formula (I) with the lactose. Fill the resulting powder blend into hard gelatin capsule shells, 30mg per capsule. Alternatively, the micronised compound of formula (I) could be compressed into a plug and a device which delivers small amounts of the compound of formula (I) into the airstream can be used.

### Nasal drops

Compound of the invention	100 mg
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Methyl p-hydroxybenzoate	10 mg
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Water for injections                    to                    10 mL

Dissolve the compound of formula (I) and the methyl p-hydroxybenzoate in the water for injections. Fill this solution into suitable dropper bottles, 10mL/bottle, and close by securing the dropper nozzle and bottle cap.

Tablet

Compound of the invention	100 mg
Lactose	100 mg
Starch	50 mg
Polyvinylpyrrolidone	5 mg
Magnesium Stearate	<u>5 mg</u>
	<u>260 mg</u>

The Compound, Lactose and Starch are mixed together and then granulated with a solution of Polyvinyl-pyrrolidone in water. After drying the granules, the Magnesium Stearate is mixed in and tablets compressed at an average weight of 260 mg.

Capsule

Compound of the invention	100 mg
Dibasic Calcium Phosphate Dihydrate	100 mg
Sodium Starch Glycolate	16 mg
Methylcellulose 400 cps	5 mg
Stearic Acid	4 mg
Talc	<u>5 mg</u>
	<u>230 mg</u>

The compound, dibasic calcium phosphate, dihydrate and sodium starch glycolate are mixed together and then granulated with a solution of the Methylcellulose in water. After drying, the granules are mixed with the Stearic Acid and Talc and the mixture filled into gelatin capsules at an average fill weight of 230 mg.

Suppository

Compound of the invention	100 mg
Suppository Base (Mixed Glycerides of saturated fatty acids)	<u>1700 mg</u>
	<u>1800 mg</u>

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Grind the Compound to a particle size below  $150\mu$ . Add the suppository base at  $38-40^{\circ}\text{C}$ . Mix to give a uniform dispersion. Pour into suppository moulds and allow to cool.

Injection - Single dose, intravenous

Compound of the invention	100 mg
Sodium Hydroxide Solution (30%)	q.s.
Water for Injections to	5 mL

Suspend the Compound in some of the Water for Injections. Adjust the pH to 10 to 10.5 by addition of Sodium Hydroxide Solution. Add sufficient Water for Injections to produce the required final volume. Re-check the pH. Sterilise by passage through a sterile membrane filter of  $0.22\mu$  pore size. Fill under aseptic conditions into sterile vials and freeze dry.

Injection - Multidose, intramuscular

Compound of the invention, sterile	1000 mg
Polysorbate 20	3 mg
Polyvinylpyrrolidone	1000 mg
Chlorocresol	60 mg
Sodium Chloride q.s. to isotonicity	
Water for Injections to	30 mL

Dissolve the Polysorbate 20, Polyvinylpyrrolidone, Sodium Chloride and Chlorocresol in Water for Injections. Sterile filter,  $0.22\mu$ . Grind the sterile Compound to a particle size below  $20\mu$  and add to the filtered solution. Mix until a uniform dispersion is achieved. Fill into sterile glass vials.

Prolonged Release Tablet

Compound of the invention	200 mg
Casein	195 mg
Hydrogenated Castor Oil	400 mg
Magnesium Stearate	<u>5 mg</u>
	<u>800 mg</u>

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Melt the Hydrogenated Castor Oil and add the Compound, ground to a particle size of less than 150 $\mu$ . Add the Casein. Mix until uniform. Allow to cool and mill to a granule. Mix in the Magnesium Stearate and compress to an average weight of 1,200 mg.

Preferred unit dosage formulations are those containing a daily dose or unit daily sub-dose, as hereinabove recited, or an appropriate fraction thereof, of a compound of the invention or physiologically acceptable salts thereof.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavouring agents.

A compound of the invention or physiologically acceptable salts thereof may also be presented as depot formulations of the kind known in the art from which the active ingredient is released, over a prolonged period, once the formulation is in place within the body of the recipient.

The results of tests with a compound of the invention are described in the following experimental examples in order to illustrate the effect of the present invention in more detail:

#### EXAMPLE 1

Mice were injected subcutaneously in the dorsal mid-line either with antigen alone, with antigen plus a conventional adjuvant, or with antigen plus test compound. Subsequent additional injections of test compound were also given where specified for up to four days at the same injection site. After seven days regional (inguinal) lymph nodes were removed and the lymph node cells restimulated with antigen alone. Antigen specific proliferation of lymphocytes was measured after a further four days by a standard procedure employing <sup>3</sup>H-thymidine uptake into DNA and liquid scintillation spectrometry. Tests for humoral immunity were performed by administering antigen and test compounds in the same way. After 1-2 weeks blood was sampled by venepuncture and serum antibody was assayed by an enzyme-linked immunosorbent (ELISA) assay.

## RESULTS

The data in figure 1 show the effects of the tucaresol on T-lymphocyte priming to antigen (keyhole limpet haemocyanin). B10S mice received 50µg of antigen either alone (■), with the conventional adjuvant alum (◇), or with 100µg of Tucaresol (◆).

The data in figure 2 show the effects of the small Schiff base-forming compound p-hydroxyphenylacetaldehyde (pHPA) (compound 1) on T-cell priming to recombinant nucleoprotein (NP) of influenza virus in B10S mice. On day 0 mice received 1µg of NP either alone (▣) or with 1mg of pHPA (■). The pHPA group received a further injection of 1mg of pHPA on days 1 and 2 (three injections in all). After 7 days regional lymph nodes were removed and the lymph node cells restimulated with A/Okuda influenza virus. Antigen specific proliferation was measured after a further 4 days as described.

The data in figure 3 show the effects of the Schiff base-forming ketones dioxoheptanoic acid (DHA) (compound 12) and 3-hydroxy-1-(4-methoxyphenyl)-3-methyl-2-butanone (HMMB) (compound 18) on the response to keyhole limpet haemocyanin (KLH) in B10S mice. A single injection of KLH (10µg) was given on day 0 and test compounds were administered at the same site on days 0, 1, 2 and 3. After 7 days regional lymph nodes were removed and LNC restimulated with antigen to measure T-cell priming in the standard procedure. Test compounds were compared with the conventional adjuvant aluminium hydroxide (100µg mixed with the antigen) as follows. Antigen alone (■). Antigen plus alum (□). Antigen plus DHA 1mg (◆). Antigen plus HMMB 2mg (◇).

The data in figure 4 show the effects of aminoguanidine, on T-cell responsiveness. This compound is a nucleophilic hydrazine which reacts readily with cellular carbonyl groups to form carbonyl-amino condensations. (The bond formed is a hydrazone, similar to a Schiff base but more stable). Mice received a single injection of KLH (50µg) either alone (▣), emulsified with Freund's complete adjuvant (◇), or with 2mg of aminoguanidine (■). T-cell priming in regional lymph nodes was assessed by the standard procedure.

The data in figure 5 show the effects of the hydrazone forming molecule pyridinium chloride hydrazide on T-cell responsiveness. Mice received a single injection of antigen (50µg of ovalbumin) either alone (▣), with 100µg of alum (◇), or with 10mg

of PCH followed by two more daily doses of 10mg PCH at the same site (■). T-cell priming was assessed by the standard procedure.

The data in figure 6 show the effects of the Schiff base forming compounds DHA and HMMB on the primary antibody response to antigen (10µg of ovalbumin) in comparison with the standard physico-chemical adjuvant alum. Antigen alone (□). Antigen plus alum (□). Antigen plus 2mg DHA (◆). Antigen plus 1mg DHA (◇), antigen plus 2mg HMMB (■).

The data in figure 7 show the effects of HMMB on the primary antibody response to KLH in B10S mice. Mice received 10µg of antigen alone (■), or antigen plus 2mg HMMB (□).

The above results show that small compounds capable of forming carbonyl-amino condensations (either Schiff bases or hydrazones) with cell-surface groups are potent enhancers of immune responses in mice.

#### EXAMPLE 2 : Antitumour Activity of Tucaresol

The activity of the tucaresol was evaluated against the outgrowth of subcutaneously implanted mouse colon adenocarcinoma 38 (MCA38) in female C57BL/6 mice, using the following protocol:

- Day 0      Fifteen, female and 16 male C57BL/6 mice each implanted subcutaneously with one 2mm cube of tumour. Mice randomized and divided into three groups - Group A, 5 females and 5 males, Group B, 5 females and 5 males, Group C, 5 females and 6 males.
- Day 5      Test groups B and C each dosed with 589C80 (1mg or 0.2mg per  
to Day 22   mouse dissolved in 0.2ml PBS, intraperitoneally) once every alternate day (total of 9 doses). Control mice (group A) given PBS alone.
- Day 24      All mice killed, tumours excised and weighed. Mean and median tumour weight in test group compared with that in controls.

Tucaresol was dissolved in PBS by dropwise addition of 1M KOH to pH 10.0 followed by dropwise addition of conc. HCL to return pH to neutral.

Dosing schedule where drug was given on alternate days was chosen in order to extend the dosing period over 14 rather than 7 days. By so doing tumours were exposed to the drug from day 13 right up until day 25 i.e. 2 days before the experiment was terminated. Results are shown in Table 1.

The pattern of tumour growth (diffuse granular) rendered calliper measurements of volume, meaningless and such data are therefore not included.

TABLE 1

## TUMOUR WEIGHT (grammes) ON DAY 24 AFTER TUMOUR IMPLANTATION

	GROUP A controls	GROUP B, 1.0mg/mouse	GROUP C, 0.2mg/mouse
	1.207	0.740	0.802
	1.130	0.695	0.272
	0.772	0.358	0.230
	0.728	0.303	0.197
	0.636	0.298	0.181
	0.575	0.090	0.126
	0.335	0.079	0.126
	0.276	0.038	0.085
	0.060	0.022	0.029
	N.T.	N.T.	0.019
			0.006
<hr/>			
Mean	0.635	0.291	0.188
S.D.	0.379	0.272	0.222
Mean % red. -		54.2	70.4
Median	0.636	0.298	0.126
Median % red. -		53.15	80.19

N.T. - no tumour take. These mice not included in derived values given above.

Tucaresol inhibited the outgrowth of subcutaneously implanted MCA38 in syngeneic C57BL/6 mice.

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EXAMPLE 3: Experimental protocol for results in Table 2

Blood from healthy donors who had been immunised within the last five years with tetanus toxoid was taken by venipuncture. The mononuclear cell fraction was prepared by density gradient centrifugation on Ficoll triosil (Lymphoprep, Nycomed AS Oslo, Norway). Cells were washed and resuspended in RPMI 1640 tissue culture medium (Flow Laboratories, Ayrshire, Scotland) containing 10% autologous serum and supplemented with antibiotics. Cells were adjusted to  $10^6$  per ml and 100 $\mu$ l aliquots were placed in each well of 96-well tissue culture microtitre plates (u-shaped wells) (Titertek, Flow Labs). Test compounds were added in 10 $\mu$ l volumes to each well and suitable vehicle controls were also added at this stage. Affinity purified tetanus toxoid was added at a final concentration of 10 $\mu$ g per ml. The cells were cultured for five days in humidified air containing 5% CO<sub>2</sub> at 37°C with <sup>3</sup>H thymidine present for the last 18 hours of culture (1 $\mu$ Ci per well). Cells were harvested onto glass microfibre paper by a semi-automatic system (Skatron) and the degree of thymidine incorporation into DNA was determined by liquid scintillation spectrometry. IC 50 ratios were determined in comparison with the benchmark compound p-hydroxyphenylacetaldehyde

The mechanism by which Schiff base forming compounds influence immune responses either positively or negatively is the same in that compounds react with amino groups on the surface of lymphocytes and antigen presenting cells (APC). In this way they provide costimulation to T-cells, amplifying the costimulation provided by physiological Schiff base-formation between ligands on the surface of cells. At high concentrations, however, the compounds will pre-empt the reaction between cellular ligands which, in addition to providing costimulation, appears to be necessary for the effective binding of APC to T-cell. Low and intermediate concentrations will thus enhance immune responses whereas high concentrations will be inhibitory. The inhibitory effect occurs more readily in vitro (because processed antigen on the surface of APC is a limiting element) and provides a measure of Schiff base forming capacity that reflects immunopotentiating capacity in vivo. Reversibility of Schiff base formation, isomerisations and cycles of protonation and deprotonation may also be important in immunopotentialiation, producing deviation from the simple equation of immunosuppression in vitro with immunopotentialiation in vivo. In vivo potency will

also be influenced by half-life, solubility, and pharmacokinetic properties of compounds.

EXAMPLE 4: A competitive assay for Schiff base formation by compounds on cell-surface  $\epsilon$ -amino groups

In order to measure the formation of a Schiff base on lymphocyte cell surface amino groups the following competitive assay for Schiff base formation on cell surface amines is used: Sulfo-NHS-biotin reacts with free amino groups on proteins to form an amide bond. Using Sulfo-NHS-biotin with an FITC (Fluorescein isothyanate)-avidin-biotin affinity labelling system cell surface amino groups are stained and measured by fluorescence activated cell sorting (FACS) analysis. Schiff base forming aldehydes and ketones competitively inhibit the reaction of Sulfo-NHS-biotin with cell surfaces, using the following assay:

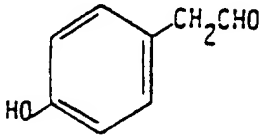
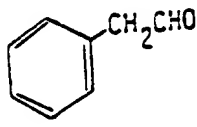
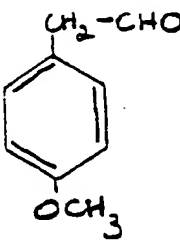
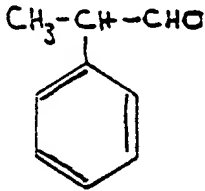
Prepare peripheral blood mononuclear cells and adjust to  $5 \times 10^6$  per ml in phosphate buffered saline. To one sample add test compound at 5mM concentration. Incubate cells for 30 minutes at 37°C. The control sample is incubated with PBS. Add  $\text{NaCNBH}_3$  (corrected to neutral pH) at 10mM for 1 hour at room temperature. Wash cells twice in PBS. Add Sulfo-NHS-biotin at 5mM final concentration for 1 hour at 37°C. Wash cells twice in PBS. Add FITC-avidin 1:50 dilution of commercial stock for 30 minutes at 4°C for 30 minutes. Wash cells three times in cold PBS. Fix with 1% paraformaldehyde. Perform FACS analysis.

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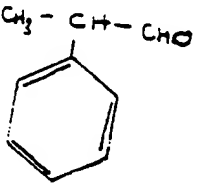
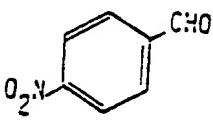
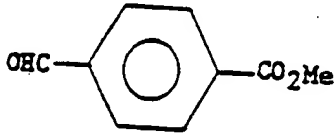
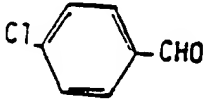
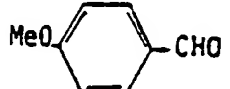


TABLE 2

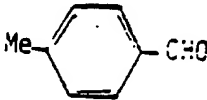
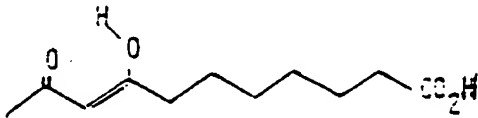
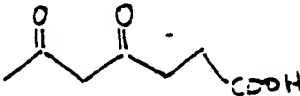
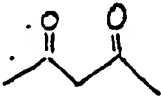
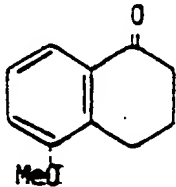
IMMUNOSUPPRESSION IN VITRO BY SCHIFF BASE FORMING  
COMPOUNDS

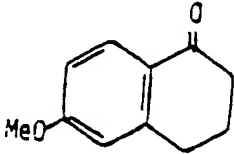
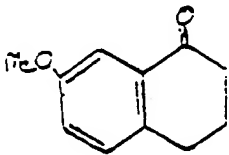
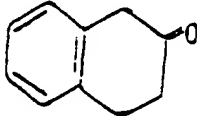
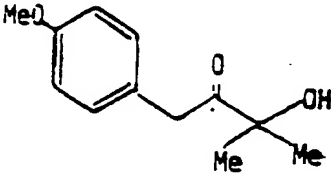
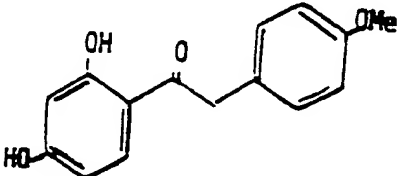
Compound Name	Structure	1C50 Ratio with 4-Hydroxyphenyl- acetaldehyde
1. 4-Hydroxyphenyl- acetaldehyde		1
2. Phenylacetaldehyde		10
3. 4-Methoxyphenyl acetaldehyde		1.4,1.9,1.9,2.3
4. Sodium 1-hydroxy-2- phenylpropane sulphonate tested as the aldehyde		30, >30

**SUBSTITUTE SHEET**

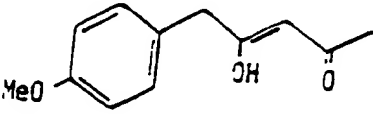
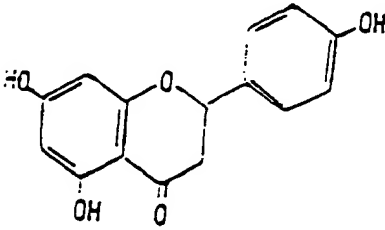
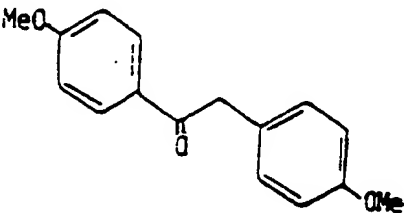
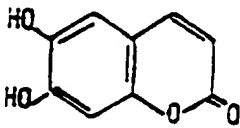
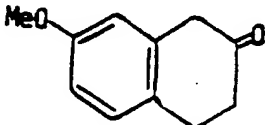
Compound Name	Structure	1C50 Ratio with 4-Hydroxyphenyl- acetaldehyde
5. 3-Phenylproponion- aldehyde		40
6. 4-Nitrobenzaldehyde		7
7. Methyl 4-formylben- zoate		8.5
8. 4-Chlorobenzaldehyde		>4
9. 4-Methoxybenzaldehyde		>4, >10

SUBSTITUTE SHEET

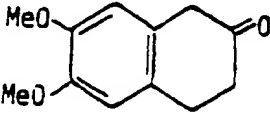
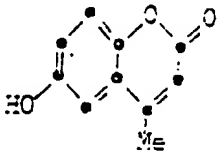
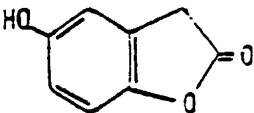
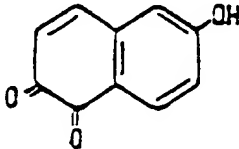
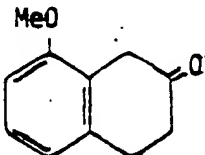
Compound Name	Structure	IC50 Ratio with 4-Hydroxyphenyl- acetaldehyde
10. 4-Methylbenzaldehyde		>5, >20
11. 8,10-Dioxoundecanoic acid		50
12. 4,6-Dioxoheptanoic acid		80
13. Pentanedione		8
14. 5-methoxy-1-tetralone		11

Compound Name	Structure	1C50 Ratio with 4-Hydroxyphenyl- acetaldehyde
15. 6-methoxy-1-tetralone		11
16. 7-methoxy-1-tetralone		16
17. 2-tetralone		2, 1
18. 3-hydroxy-1-(4-methoxy-phenyl)-3-methyl-2-butanone		0,34, 2.0, 1.0
19. 2',4'-dihydroxy-2-(4-methoxyphenyl)acetophenone		2.7

SUBSTITUTE SHEET

Compound Name	Structure	1C50 Ratio with 4-Hydroxyphenyl- acetaldehyde
20. 2-hydroxy-1-(4-methoxy-phenyl)-pent-2-ene-4-one		4, 6.6
21. Naringenin 4',5,6-tri-hydroxyflavonone		1, 5
22. 4'-methoxy-2-(4-methoxy-phenyl)acetophenone		5.5
23. 6,7-dihydroxycoumarin		2.3
24. 7-methoxy-2-tetralone		3.0

SUBSTITUTE SHEET

Compound Name	Structure	1C50 Ratio with 4-Hydroxyphenyl- acetaldehyde
25. 6,7-dimethoxy-2-tetra- lone		2.7
26. 6-hydroxy-4-methylcou- marin		2, 2.5
27. Homogentisic acid gamma lactone		.25, 0.5, 0.7, 1.5
28. 6-hydroxy-1,2-naphtho- quinone		0.2, 0.5, 1.0
29. 8-methoxy-2-tetralone		0.8, 1.0

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CLAIMS

- 1) Use of a compound or physiologically acceptable salts thereof which forms a Schiff base or a hydrazone with T-cell surface carbonyl or amino groups, with the proviso that the compound cinnaldehyde is excluded, for the manufacture of a medicament for the potentiation of an immune response.
- 2) Use according to claim 1 of a compound of formula (I) :



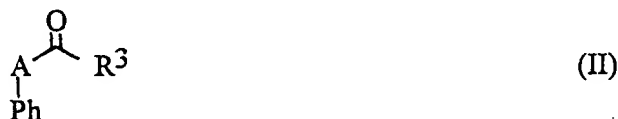
wherein R is hydrogen, methyl optionally substituted by a substituted phenyl, or by two C<sub>1-4</sub> alkyl groups and a hydroxy group;

R<sup>1</sup> is a group CH<sub>2</sub>COR<sup>2</sup> or the tautomeric form thereof wherein R<sup>2</sup> is methyl optionally substituted by a group CO(CH<sub>2</sub>)<sub>x</sub>CO<sub>2</sub>H wherein x is 1 to 7;

or R<sup>1</sup> is a group A-Ph where A is O, CH<sub>2</sub>, CH(CH<sub>3</sub>), CO or a single bond and Ph is an optionally substituted phenyl group;

or R is linked to R<sup>1</sup> to form a nine or ten-membered optionally substituted bicyclic ring system of which one ring is benzene or a physiologically acceptable salt thereof.

- 3) Use according to either claim 1 or claim 2 where a subclass of formula I is represented by a compound of formula (II)



wherein A and Ph are as hereinbefore defined;

- 38 -

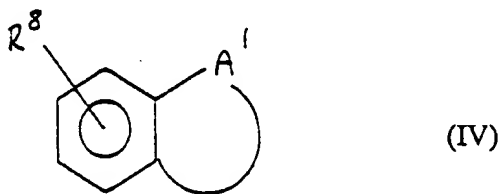
$R^3$  is H,  $C_{1-4}$  alkyl or  $CH_2(C_6H_4)R^4$ , wherein  $R^4$  is hydroxy or  $C_{1-4}$  alkoxy or a physiologically acceptable salt thereof.

- 4) Use according to any one of claims 1 to 3 where a subclass of formula (I) is represented by a compound of formula (III)



wherein  $R^7$  is a group  $CH_2COR^2$  where  $R^2$  is hereinbefore defined or a physiologically acceptable salt thereof.

- 5) Use according to any one of claims 1 to 4 wherein the bicyclic ring system is of formula (IV)

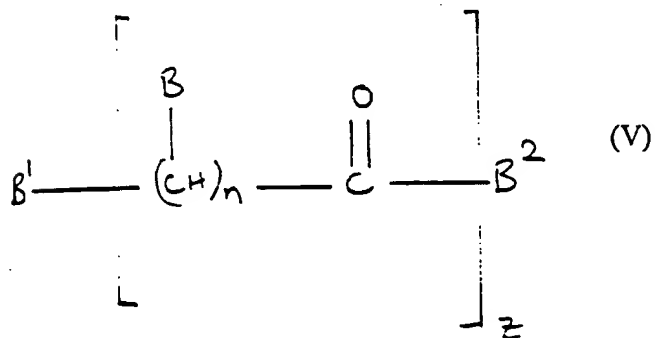


wherein  $R^8$  is hydroxy or  $C_{1-4}$  alkoxy;

$A^1$  is O, CO or  $CH_2$ ;

— represents  $C_{2-3}$  alkylene optionally substituted by a phenol group or a physiologically acceptable salt thereof.

- 6) Use of a compound of formula (V)



**SUBSTITUTE SHEET**



- 39 -

or a physiologically acceptable salt thereof, for the potentiation of an immune response, wherein

$z = 0$  or  $1$ ;

$n$  is  $0$  or  $1$ ;

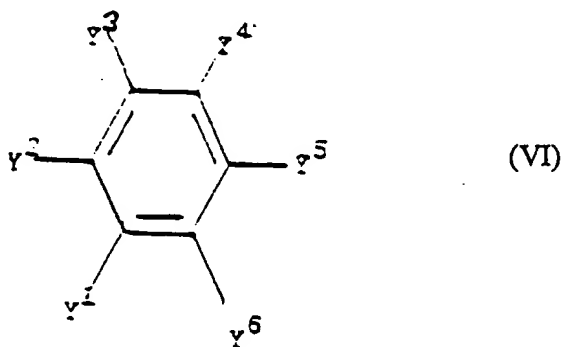
$B$  is hydrogen or  $C_{1-4}$  alkyl;

$B^1$  is hydrogen, or an optionally substituted phenyl;

$B^2$  is hydrogen, methyl, methyl optionally substituted by a group  $CO(CH_2)_xCO_2H$  wherein  $x$  is  $1$  to  $7$  or  $B^2$  is an oxygen or a  $C_{2-3}$  alkylene group optionally containing an oxygen linked to  $B^1$  when  $B^1$  is phenyl to form an optionally substituted bicyclic ring system containing  $9$  or  $10$  atoms of which one may be oxygen and the rest carbon;

when  $z = 0$ ;  $B^1$  cannot be hydrogen and  $B^2$  must be hydrogen.

7. Use according to any one of claims 1 to 3 and 6 wherein a preferred group of compounds is represented by formula (VI)



wherein

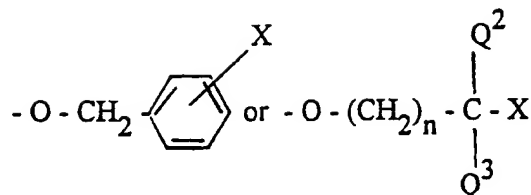
$Y^1$  is selected from hydrogen, hydroxy,  $C_{1-4}$  alkylamino, and alkanoylamino having  $1$  to  $4$  carbon atoms in the alkyl moiety thereof;

$Y^2$ ,  $Y^3$  and  $Y^4$  are independently selected from hydrogen, halogen,  $C_{1-4}$  alkyl,  $C_{1-4}$  alkoxy, haloalkyl, hydroxyl and benzyloxy; and

$Y^5$  is hydroxyl or a group  $Q^1$  wherein  $Q^1$  is either

**SUBSTITUTE SHEET**

- 40 -



where

$Q^2$  and  $Q^3$  are independently selected from hydrogen and  $C_{1-4}$  alkyl;

X is selected from cyano, carboxyl or a functional carboxylic acid derivative thereof, 5-tetrazolyl and alkylsulphonylcarbamoyl having 1 to 6 carbon atoms in the alkyl moiety thereof; and n is 0 to 6;

$Y^6$  is  $-(C_pH_{2p})CHO$ , wherein  $p = 0$  to 5

when p is > 1 the formyl(-CHO) in group  $Y^6$  may be on any carbon atom and the alkylene ( $C_pH_{2p}$ ) may be linear or branched;

or  $Y^6$  is  $(CH_2CO)_q-X^1$  or  $(COCH_2)_q-X^1$  where  $q = 1$  to 3 and  $X^1$  is methoxyphenyl, or  $C_{1-4}$  alkyl or a physiologically acceptable salt thereof.

8. Use according to claim 7 wherein

X is selected from cyano, 5-tetrazolyl, alkylsulphonylcarbamoyl having 1 to 6 carbon atoms in the alkyl moiety thereof and a group -CO.Y, where

Y is  $-OR^9$  and  $R^9$  is hydrogen, alkyl of 1 to 4 carbon atoms or benzyl, or

Y is  $-NR^{10}R^{11}$  where  $R^{10}$  and  $R^{11}$  are independently hydrogen or alkyl of 1 to 4 carbon atoms,

or a physiologically acceptable salt thereof.

9. Use according to any one of claims 6 to 8 wherein X is carboxyl, together with physiologically acceptable salts thereof.

**SUBSTITUTE SHEET**

- 41 -

10. Use according to claim 6 wherein  $B_1$  and  $B_2$  form a bicyclic ring system which is selected from coumarin, tetralone, benzofuranone or naphthoquinone substituted by up to four substituents selected from hydrogen,  $C_{1-4}$  alkoxy, hydroxy or phenyl substituted by hydroxy or a physiologically acceptable salt thereof.
11. Use of 4-(2-formyl-3-hydroxyphenoxymethyl)benzoic acid or a physiologically acceptable salt thereof for the manufacture of a medicament for the potentiation of an immune response.
12. Use of a compound or a physiologically acceptable salt thereof as defined in any one of claims 1 to 11 in the manufacture of a medicament for the treatment and/or prophylaxis of a viral infection.
13. Use according to claim 12 wherein the virus infection is a Human Immunodeficiency Virus (HIV) infection.
14. Use according to claim 12 wherein the virus infection is a hepatitis B infection.
15. Use according to claim 12 wherein the virus infection is a hepatitis C infection.
16. Use of a compound or a physiologically acceptable salt thereof as defined in any one of claims 1 to 11 in the manufacture of a medicament for the treatment and/or prophylaxis of cancer.
17. Use according to claim 16 wherein the cancer is colorectal cancer..
18. Use of a compound or a physiologically acceptable salt thereof as defined in any one of claims 1 to 17, further containing other chemotherapeutic agent and/or another immunotherapeutic agent.
19. Use of a compound or a physiologically acceptable salt thereof as defined in any one of claims 1 to 18, suitable for nasal or pulmonary administration.
20. A method for treating an immunodeficient mammal which comprises administering to said mammal an effective amount of a compound or a physiologically acceptable salt thereof which forms a Schiff base or a hydrazone with T-cell surface carbonyl or amino groups, with the proviso that the compound cinnaldehyde is excluded, for the potentiation of an immune response.

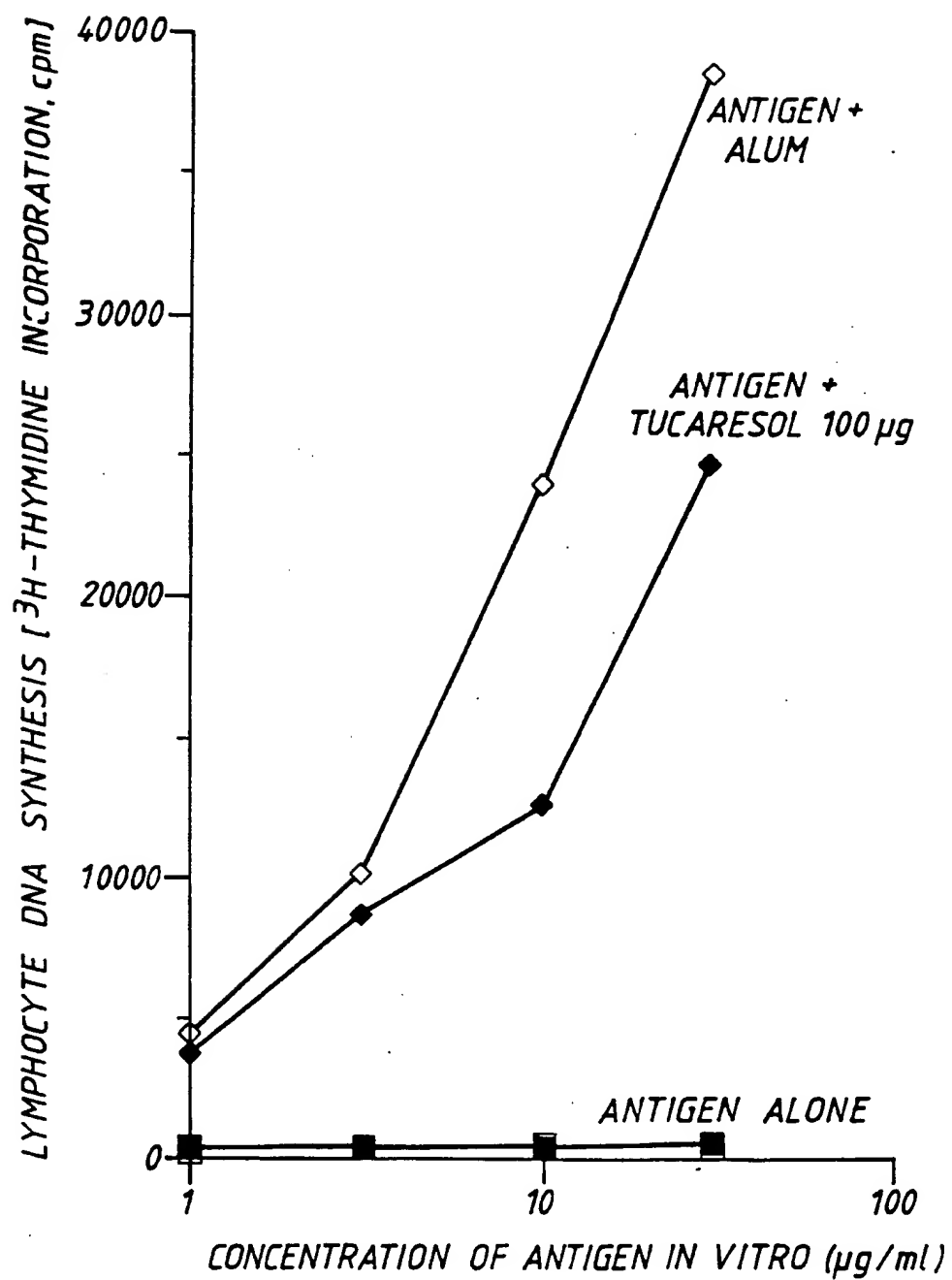
**SUBSTITUTE SHEET**

- 42 -

21. A method for treating an immunodeficient mammal, which comprises administering to said mammal an effective immunopotentiatory amount of 4-(2-formyl-3-hydroxyphenoxyethyl)benzoic acid or a physiologically acceptable salt thereof, for the potentiation of an immune response.
22. A method according to claim 21 wherein the effective immunopotentiatory amount of 4-(2-formyl-3-hydroxyphenoxyethyl)benzoic acid or a physiologically acceptable salt thereof, is administered as a daily dose for at least five days.
23. A method for the treatment of virus infections in a mammal whose immune system is depressed which comprises administering to the mammal in need of treatment a therapeutically effective amount of 4-(2-formyl-3-hydroxyphenoxyethyl)benzoic acid or a physiologically acceptable salt thereof.
24. A method according to claim 23 wherein the virus infection is a Human Immunodeficiency Virus (HIV) infection.
25. A method according to claim 23 wherein the virus infection is a hepatitis B virus.
26. A method according to claim 23 wherein the virus infection is a hepatitis C virus.
27. A method for the treatment of cancer in a mammal which comprises administering to the mammal in need of treatment a therapeutically effective amount of 4-(2-formyl-3-hydroxyphenoxyethyl)benzoic acid or a physiologically acceptable salt thereof.
28. A method according to claim 27 wherein the cancer is colorectal cancer.
29. A method according to claim 27 wherein the cancer is malignant melanoma.
30. A method according to claim 27 wherein the cancer is renal cancer.
31. A method according to any one of claims 20-30 wherein the effective amount is from 50 to 200 mg per day.

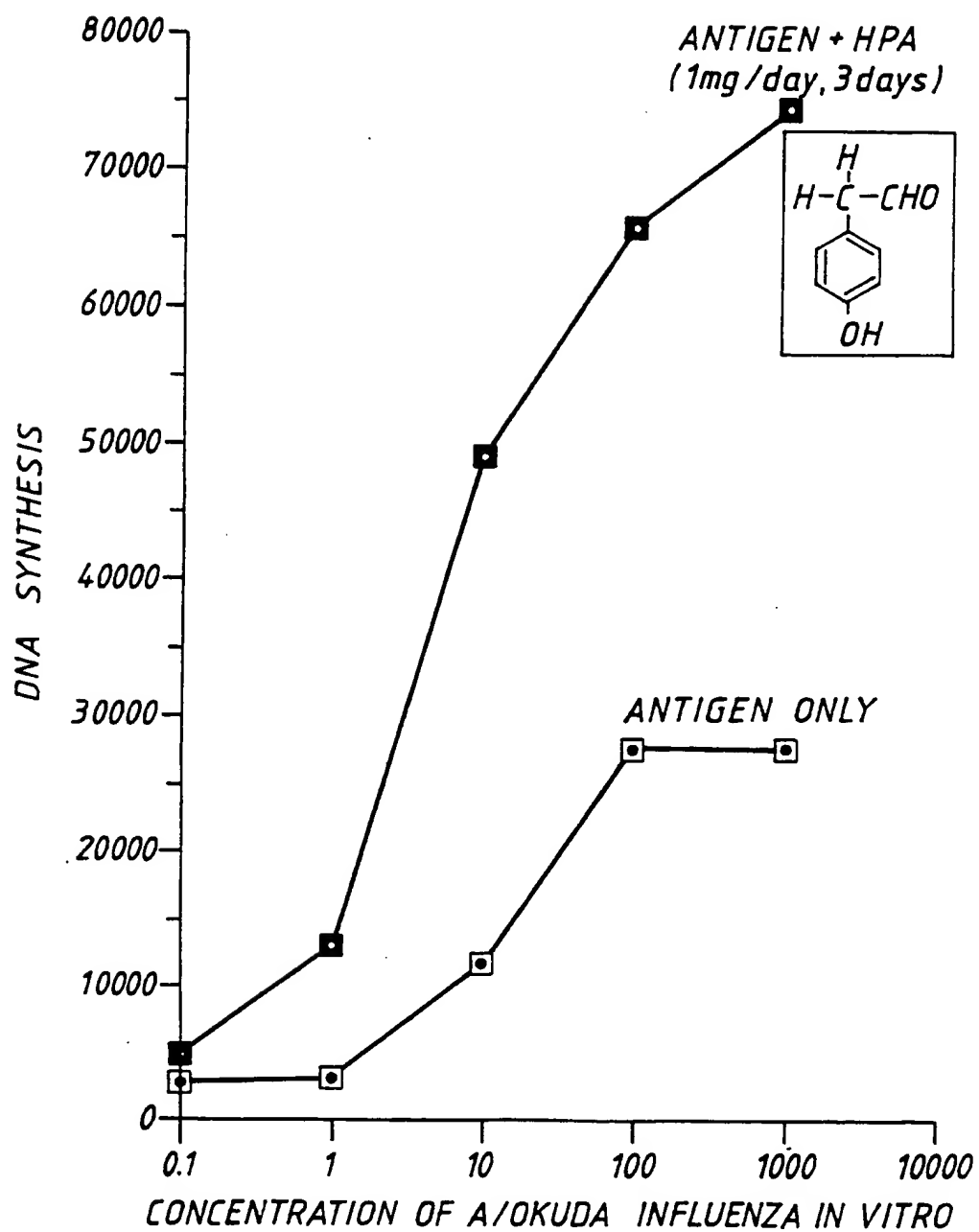
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FIG. 1



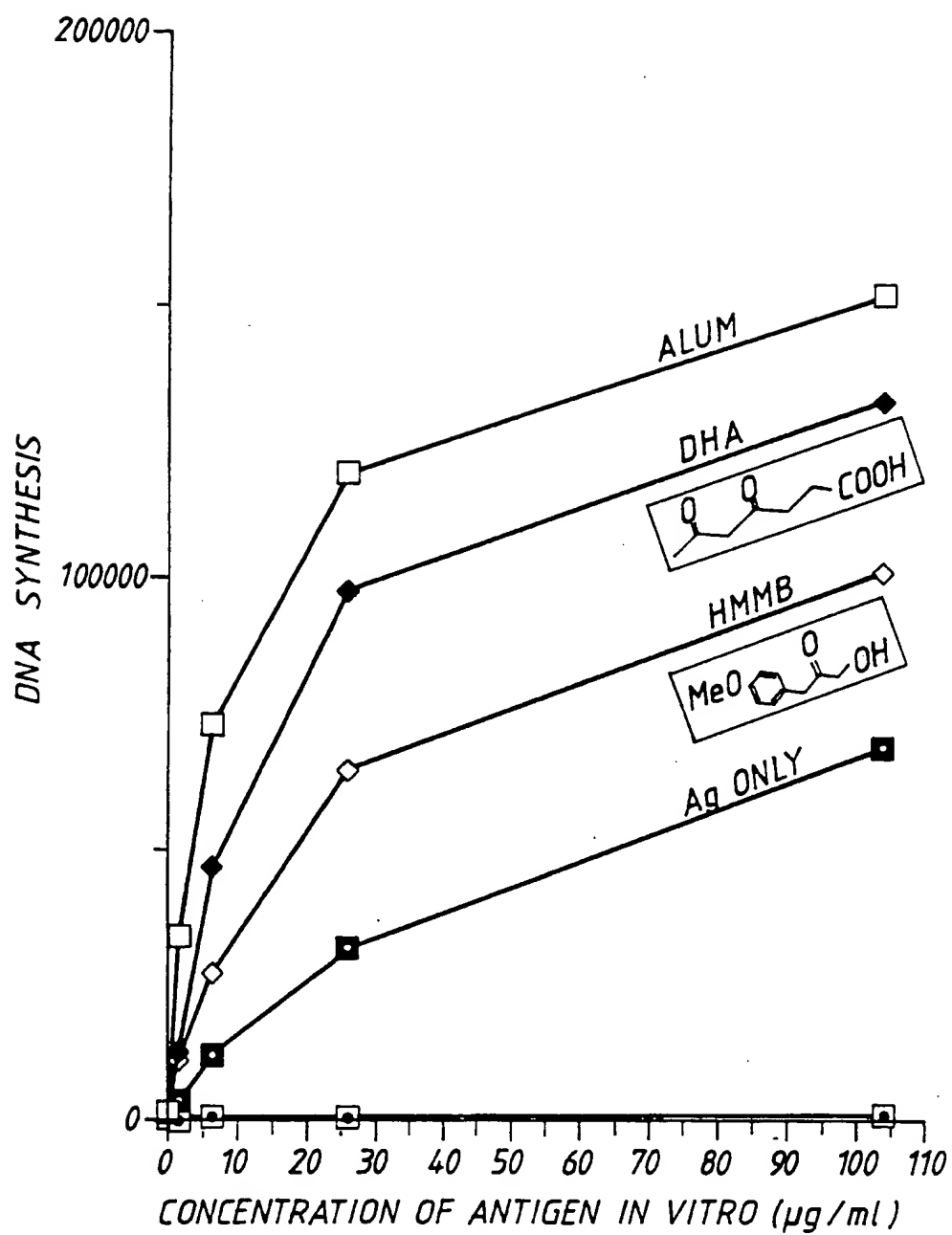
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FIG. 2



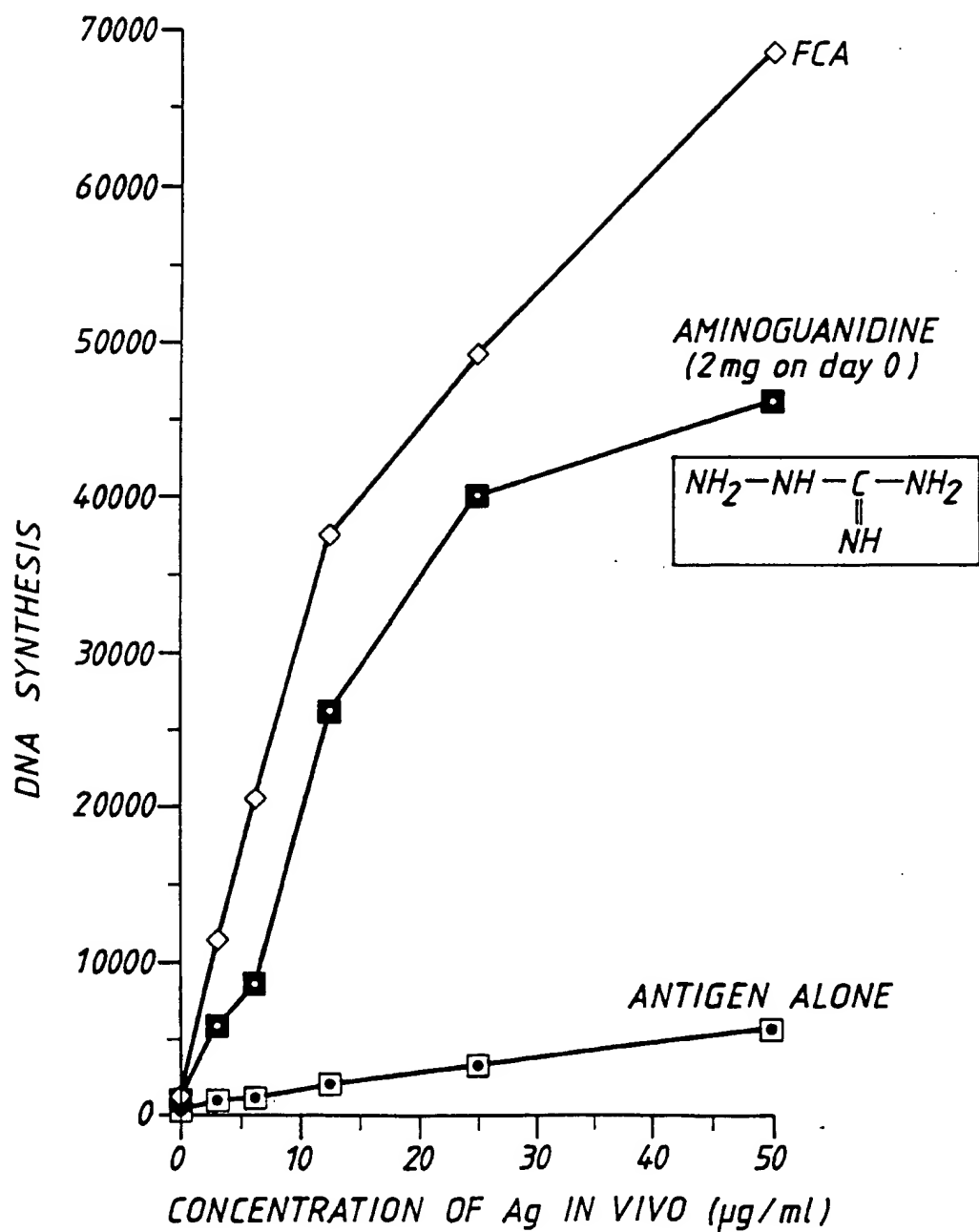
3/7

FIG. 3



4/7

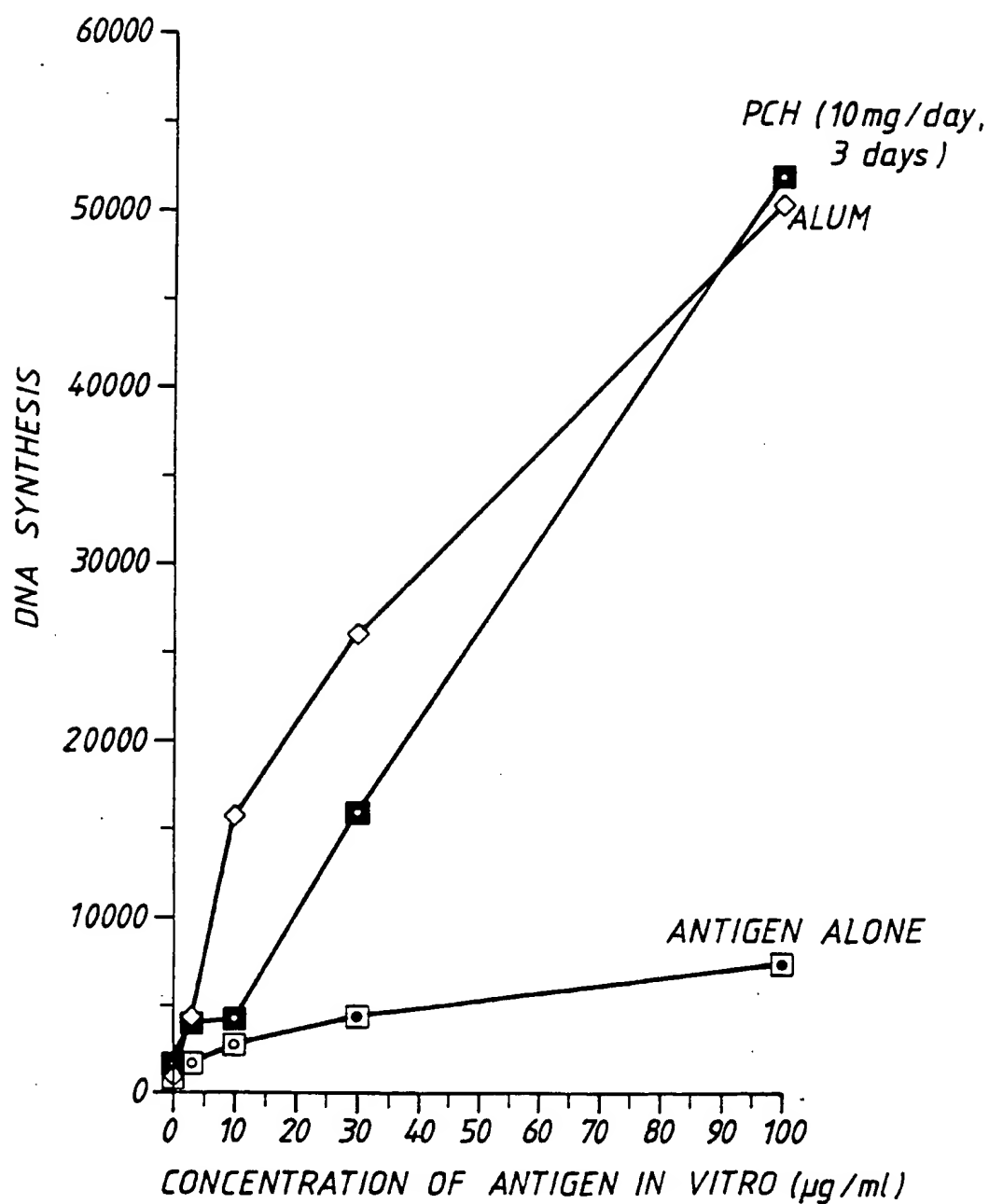
FIG. 4





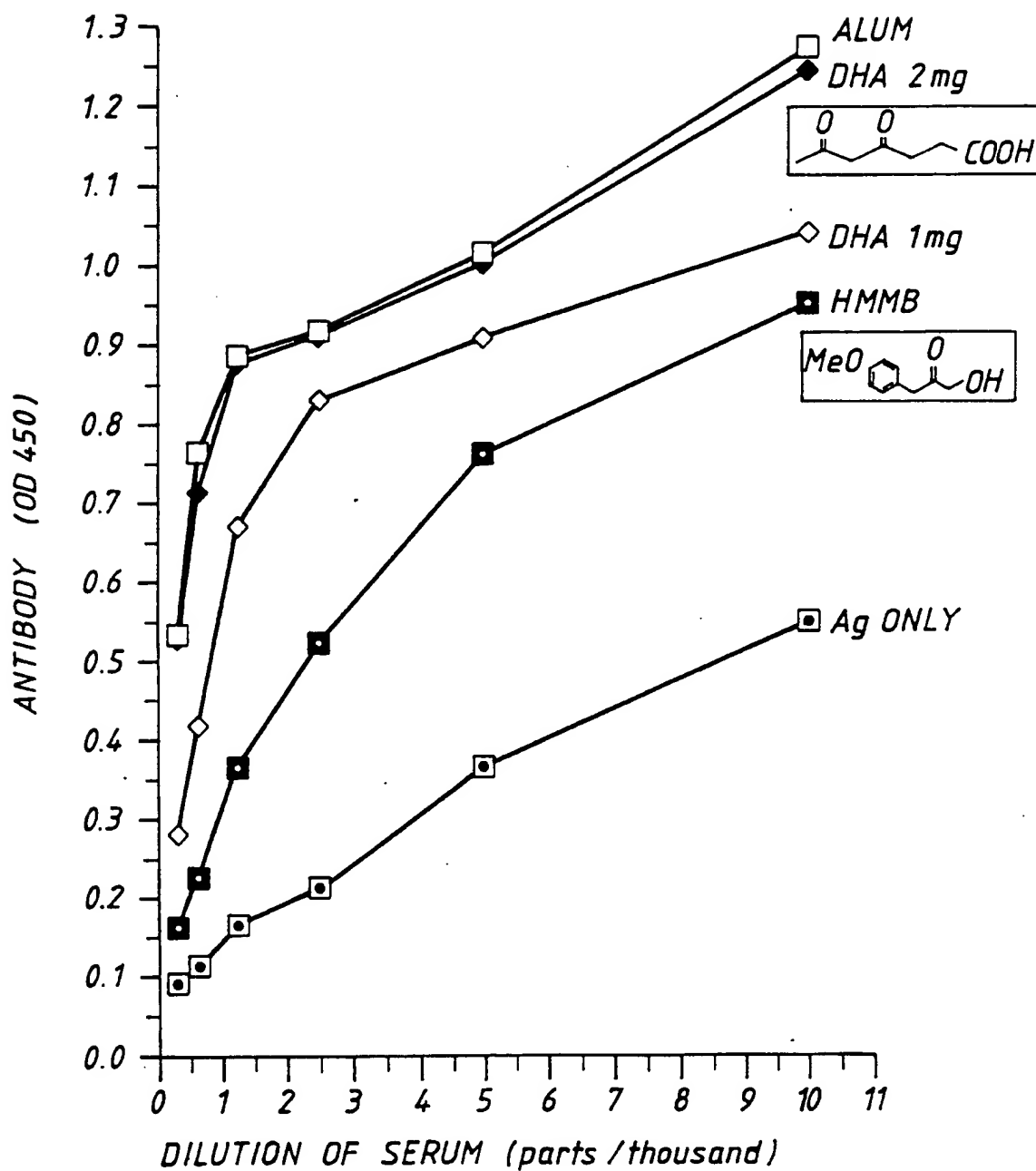
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FIG. 5



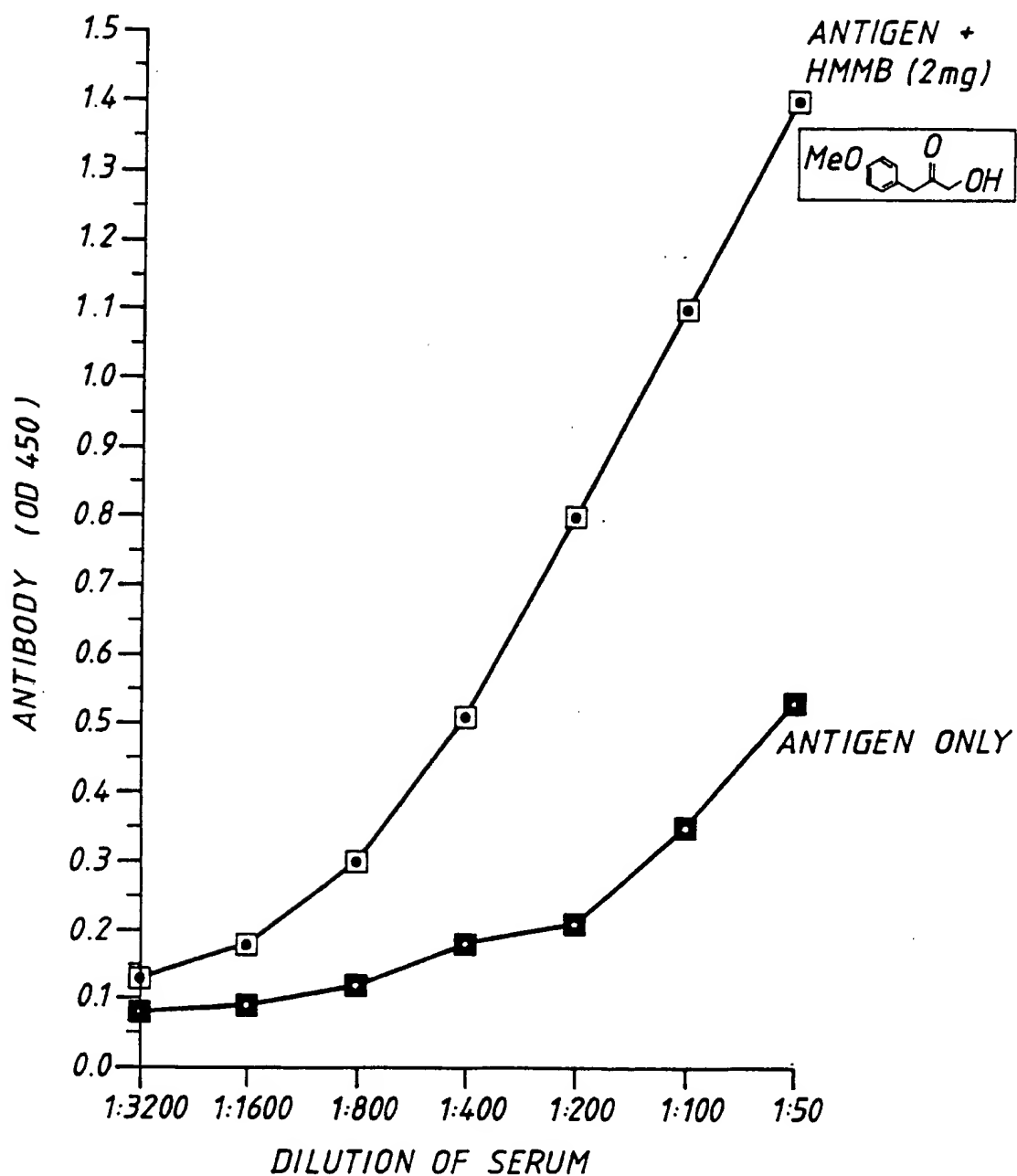
6/7

FIG. 6



7/7

FIG. 7



## INTERNATIONAL SEARCH REPORT

International Application No

PC/GB 93/02039

A. CLASSIFICATION OF SUBJECT MATTER  
 IPC 5 A61K31/115 A61K31/12 A61K31/365 A61K31/37 A61K31/41

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP-A-0 173 262 (CARNIVORA-DEUTSCHLAND) 5 March 1986 see abstract; claims 1,2 ---	1,2,5, 10,12-19
X	EP-A-0 499 015 (FOCKERMANN) 19 August 1992  see abstract ---	1,2,5, 10,12-19
X	EP-A-0 217 041 (SCHLEICHER) 8 April 1987  see column 1, line 47 - column 2, line 20 --- -/--	1,2,5, 10,12-19

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*&\* document member of the same patent family

Date of the actual completion of the international search

17 January 1994

Date of mailing of the international search report

29.03.94

Name and mailing address of the ISA

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Authorized officer

Foerster, W

## INTERNATIONAL SEARCH REPORT

International Application No

PC/GB 93/02039

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE WPI  Week 8106,  Derwent Publications Ltd., London, GB;  AN 81-003823  &amp; RO-A-73 330 (TERAPIA INTR MED) 31 March  1980  see abstract</p> <p>---</p>	1,2,5, 10,12-19
X	<p>PATENT ABSTRACTS OF JAPAN  vol. 004, no. 081 (C-014) 28 September  1978  &amp; JP-A-55 045 658 (RIKAGAKU KENKYUSHO) 31  March 1980  see abstract</p> <p>---</p>	1,2
X	<p>PATENT ABSTRACTS OF JAPAN  vol. 013, no. 072 (C-570) 17 February 1989  &amp; JP-A-63 264 409 (RIKAGAKU KENKYUSHO) 1  November 1988  see abstract</p> <p>---</p>	1,2
X	<p>PATENT ABSTRACTS OF JAPAN  vol. 004, no. 106 (C-020) 30 July 1980  &amp; JP-A-55 069 510 (MITSUBISHI CHEM. IND.  LTD.) 26 May 1980  see abstract</p> <p>---</p>	1,2
X	<p>PATENT ABSTRACTS OF JAPAN  vol. 003, no. 147 (C-066) 5 December 1979  &amp; JP-A-54 126 731 (RIKAGAKU KENKYUSHO) 2  October 1979  see abstract</p> <p>-----</p>	1,2

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB93/02039

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 20-31  
because they relate to subject matter not required to be searched by this Authority, namely:  
Please see Rule 39.1(iv) PCT!
2. ☒ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
The following references to claims appear to be wrong or unclear: to claim 3 in claim 4, to claim 3 and 4 in claim 5, to claim 6 in claim 7, to claim 6 in claim 9. (CLAIMS SEARCHED INCOMPLETELY: 4;5;7:9)
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

For further information please see form PCT/ISA/206 dated 02.02.1994.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1,2,5,10 and 12-19 (partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/GB 93/02039

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0173262	05-03-86	DE-A- 3431236	27-02-86
EP-A-0499015	19-08-92	AU-A- 1277992	15-09-92
		EP-A- 0571491	01-12-93
		WO-A- 9214458	03-09-92
EP-A-0217041	08-04-87	DE-A- 3528709	19-02-87
		JP-A- 62039516	20-02-87